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	DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI; PLUR=YES; OP=ADJ		
☐	L9	growth hormone NEAR purification	29
☐	L8	s purification near growth hormone	0
☐	L7	l6 and cell concentrate	4
☐	L6	L5 and purification	11059
☐	L5	L4 and sodium	11895
☐	L4	L3 and ph	13574
☐	L3	L2 and recombinant	14527
☐	L2	L1 and yeast	15690
☐	L1	growth hormone	34983

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      (FILE 'HOME' ENTERED AT 06:58:02 ON 24 JAN 2007)

      FILE 'MEDLINE, EMBASE, BIOSIS' ENTERED AT 06:58:17 ON 24 JAN 2007
L1        183967 S GROWTH HORMONE OR GH OR HGH OR SOMATOTRO?
L2           336 S L1 AND YEAST
L3             1 S L2 AND SALT
L4           102 S L2 AND RECOMBINANT
L5             8 S L4 AND PH
L6             8 DUPLICATE REMOVE L5 (0 DUPLICATES REMOVED)

      FILE 'STNGUIDE' ENTERED AT 07:00:23 ON 24 JAN 2007

      FILE 'MEDLINE, EMBASE, BIOSIS' ENTERED AT 07:02:08 ON 24 JAN 2007
L7             8 S L6
L8             3 S L2 AND POTASSIUM
L9            11 S L2 AND SODIUM
L10            5 DUPLICATE REMOVE L9 (6 DUPLICATES REMOVED)
L11           33 S L2 AND PURIFI?
L12           21 DUPLICATE REMOVE L11 (12 DUPLICATES REMOVED)

      FILE 'STNGUIDE' ENTERED AT 07:06:57 ON 24 JAN 2007
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L12 ANSWER 1 OF 21 MEDLINE on STN DUPLICATE 1
AN 2006115355 MEDLINE
DN PubMed ID: 16491466
TI Construction of a protease-deficient strain set for the fission yeast Schizosaccharomyces pombe, useful for effective production of protease-sensitive heterologous proteins.
AU Idiris Alimjan; Bi Kewei; Tohda Hideki; Kumagai Hiromichi; Giga-Hama Yuko
CS ASPEX Division, Research Centre, Asahi Glass Co. Ltd, 1150 Hazawa-cho, Kanagawa-ku, Yokohama 221-8755, Japan.
SO Yeast (Chichester, England), (2006 Jan 30) Vol. 23, No. 2, pp. 83-99. Journal code: 8607637. ISSN: 0749-503X.
CY England: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200604
ED Entered STN: 28 Feb 2006
Last Updated on STN: 19 Apr 2006
Entered Medline: 18 Apr 2006

L12 ANSWER 2 OF 21 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
AN 2006:247364 BIOSIS
DN PREV200600248351
TI Cloning and expression of novel somatotropin gene in bacterial (E-coli) and yeast (Pichia pastoris) expression systems.
AU Sadaf, S. [Reprint Author]; Damasceno, L. M.; Wilson, D. B.; Akhtar, M. W.
CS Univ Punjab, Inst Biochem and Biotechnol, Lahore, Pakistan
sasadaf@hotmail.com
SO FEBS Journal, (JUL 2005) Vol. 272, No. Suppl. 1, pp. 518. Meeting Info.: 30th Congress of the Federation-of-European-Biochemical-Societies (FEBS)/9th IUBMB Conference. Budapest, HUNGARY. July 02 -07, 2005. Federat European Biochem Soc; Int Union Biochem Mol Biol. ISSN: 1742-464X. E-ISSN: 1742-4658.
DT Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LA English
ED Entered STN: 26 Apr 2006
Last Updated on STN: 26 Apr 2006

L12 ANSWER 3 OF 21 EMBASE COPYRIGHT (c) 2007 Elsevier B.V. All rights reserved on STN
AN 2004488557 EMBASE
TI Current and future considerations for the new classes of biologicals.
AU Kleinberg M.; Mosdell K.W.
CS Dr. K.W. Mosdell, Amgen, Mailstop 27-1-D, One Amgen Center Drive, Thousand Oaks, CA 91320, United States. mosdellk@amgen.com
SO American Journal of Health-System Pharmacy, (1 Apr 2004) Vol. 61, No. 7, pp. 695-710.
Refs: 79
ISSN: 1079-2082 CODEN: AHSPEK
CY United States
DT Journal; General Review
FS 036 Health Policy, Economics and Management
037 Drug Literature Index
039 Pharmacy
LA English
SL English
ED Entered STN: 2 Dec 2004
Last Updated on STN: 2 Dec 2004

L12 ANSWER 4 OF 21 MEDLINE on STN
AN 2004270169 MEDLINE
DN PubMed ID: 15169649

TI Construction,identification and amplification of a yeast two-hybrid random cycle peptide library.
AU Xu Xiang; Liang Hua-ping; Wang Fu-long; Luo Yan; Wang Zheng-guo
CS Research Institute of Surgery, Third Military Medical University, Chongqing 400042, China.. xuxiang75@cta.cq.cn
SO Xi bao yu fen zi mian yi xue za zhi = Chinese journal of cellular and molecular immunology, (2003 Sep) Vol. 19, No. 5, pp. 437-9. Journal code: 101139110. ISSN: 1007-8738.
CY China
DT Journal; Article; (JOURNAL ARTICLE)
LA Chinese
FS Priority Journals
EM 200406
ED Entered STN: 1 Jun 2004
Last Updated on STN: 1 Jul 2004
Entered Medline: 30 Jun 2004

L12 ANSWER 5 OF 21 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
AN 2003:94997 BIOSIS
DN PREV200300094997
TI Expression of common carp growth hormone in the yeast Pichia pastoris and growth stimulation of juvenile tilapia (Oreochromis niloticus).
AU Li, Yinghua; Bai, Junjie [Reprint Author]; Jian, Qing; Ye, Xing; Lao, Haihua; Li, Xinhui; Luo, Jianren; Liang, Xufang
CS Key Laboratory of Tropical and Subtropical Fish Breeding and Cultivation, Pearl River Fisheries Research Institute, CAFS, Ministry of Agriculture P.R.C., Guangzhou, 510380, China
jjbai@163.net
SO Aquaculture, (10 February 2003) Vol. 216, No. 1-4, pp. 329-341. print. ISSN: 0044-8486 (ISSN print).
DT Article
LA English
OS DDBJ-AF332594; EMBL-AF332594; GenBank-AF332594
ED Entered STN: 12 Feb 2003
Last Updated on STN: 4 Apr 2003

L12 ANSWER 6 OF 21 EMBASE COPYRIGHT (c) 2007 Elsevier B.V. All rights reserved on STN DUPLICATE 2
AN 2003204395 EMBASE
TI Synthesis and chromatographic purification of recombinant human pituitary hormones.
AU Ribela M.T.C.P.; Gout P.W.; Bartolini P.
CS M.T.C.P. Ribela, Biotechnology Department, IPEN-CNEN, Cidade Universitaria, Travessa R 400, 05508-900 Sao Paulo, Brazil.
mtribela@ipen.br
SO Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences, (25 Jun 2003) Vol. 790, No. 1-2, pp. 285-316. .
Refs: 236
ISSN: 1570-0232 CODEN: JCBAAI
CY Netherlands
DT Journal; General Review
FS 003 Endocrinology
029 Clinical Biochemistry
LA English
SL English
ED Entered STN: 5 Jun 2003
Last Updated on STN: 5 Jun 2003

L12 ANSWER 7 OF 21 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
AN 2001:481752 BIOSIS
DN PREV200100481752
TI Role of Rac1 in regulated exocytosis.
AU Li, Q. W. [Reprint author]; Marinescu, V. [Reprint author]; Bhatti, H.

[Reprint author]; Holz, R. W.; Stuenkel, E. L. [Reprint author]
CS Dept. of Physiology, University of Michigan, Ann Arbor, MI, USA
SO Society for Neuroscience Abstracts, (2001) Vol. 27, No. 1, pp. 107. print.
Meeting Info.: 31st Annual Meeting of the Society for Neuroscience. San Diego, California, USA. November 10-15, 2001.
ISSN: 0190-5295.
DT Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LA English
ED Entered STN: 17 Oct 2001
Last Updated on STN: 23 Feb 2002

L12 ANSWER 8 OF 21 MEDLINE on STN DUPLICATE 3
AN 2000429269 MEDLINE
DN PubMed ID: 10900040
TI Natural infection with herpes simplex virus type 1 (HSV-1) induces humoral and T cell responses to the HSV-1 glycoprotein H:L complex.
AU Westra D F; Verjans G M; Osterhaus A D; van Kooij A; Welling G W; Scheffer A J; The T H; Welling-Wester S
CS Department of Medical Microbiology, University of Groningen, Hanzeplein 1, 9713 GZ Groningen, The Netherlands.
SO The Journal of general virology, (2000 Aug) Vol. 81, No. Pt 8, pp. 2011-5.
Journal code: 0077340. ISSN: 0022-1317.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200009
ED Entered STN: 22 Sep 2000
Last Updated on STN: 12 Feb 2002
Entered Medline: 14 Sep 2000

L12 ANSWER 9 OF 21 MEDLINE on STN DUPLICATE 4
AN 1999445428 MEDLINE
DN PubMed ID: 10514258
TI Novel secretion system of recombinant Saccharomyces cerevisiae using an N-terminus residue of human IL-1 beta as secretion enhancer.
AU Lee J; Choi S I; Jang J S; Jang K; Moon J W; Bae C S; Yang D S; Seong B L
CS Biochemical Process Engineering R.U., Korea Research Institute of Bioscience and Biotechnology (KRIBB), P.O. Box 115, Yusong, Taejon 305-600, Korea.
SO Biotechnology progress, (1999 Sep-Oct) Vol. 15, No. 5, pp. 884-90.
Journal code: 8506292. ISSN: 8756-7938.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199911
ED Entered STN: 11 Jan 2000
Last Updated on STN: 11 Jan 2000
Entered Medline: 15 Nov 1999

L12 ANSWER 10 OF 21 MEDLINE on STN DUPLICATE 5
AN 97275436 MEDLINE
DN PubMed ID: 9129313
TI Role of high-performance liquid chromatographic protein analysis in developing fermentation processes for recombinant human growth hormone, relaxin, antibody fragments and lymphotoxin.
AU Jacobson F S; Hanson J T; Wong P Y; Mulkerrin M; Deveney J; Reilly D; Wong S C
CS Department of Fermentation and Cell Culture Process Development, Genentech, Inc., South San Francisco, CA 94080, USA.
SO Journal of chromatography. A, (1997 Feb 28) Vol. 763, No. 1-2, pp. 31-48.
Journal code: 9318488. ISSN: 0021-9673.

CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199706
ED Entered STN: 12 Jun 1997
Last Updated on STN: 12 Jun 1997
Entered Medline: 5 Jun 1997

L12 ANSWER 11 OF 21 MEDLINE on STN DUPLICATE 6
AN 96422955 MEDLINE
DN PubMed ID: 8825556
TI Characterization of the cyclic adenosine 3',5'-monophosphate response element of the rabbit surfactant protein-A gene: evidence for transactivators distinct from CREB/ATF family members.
AU Michael L F; Alcorn J L; Gao E; Mendelson C R
CS Department of Biochemistry, University of Texas Southwestern Medical Center at Dallas 75235-9038, USA.
NC HL50022 (NHLBI)
SO Molecular endocrinology (Baltimore, Md.), (1996 Feb) Vol. 10, No. 2, pp. 159-70.
Journal code: 8801431. ISSN: 0888-8809.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199612
ED Entered STN: 28 Jan 1997
Last Updated on STN: 18 Dec 2002
Entered Medline: 4 Dec 1996

L12 ANSWER 12 OF 21 MEDLINE on STN
AN 95048329 MEDLINE
DN PubMed ID: 7959728
TI Isolation of cosmid and cDNA clones in the region surrounding the BTK gene at Xq21.3-q22.
AU Vorechovsky I; Vetrie D; Holland J; Bentley D R; Thomas K; Zhou J N; Notarangelo L D; Plebani A; Fontan G; Ochs H D; +
CS Center for BioTechnology, Karolinska Institute at NOVUM, Huddinge, Sweden.
SO Genomics, (1994 Jun) Vol. 21, No. 3, pp. 517-24.
Journal code: 8800135. ISSN: 0888-7543.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-L35265; GENBANK-U01922; GENBANK-U01923; GENBANK-U01925
EM 199411
ED Entered STN: 10 Jan 1995
Last Updated on STN: 29 Jan 1996
Entered Medline: 30 Nov 1994

L12 ANSWER 13 OF 21 MEDLINE on STN
AN 95140897 MEDLINE
DN PubMed ID: 7838974
TI Study of Bacillus sp. culture conditions to promote production of unhairing proteases.
AU Loperena L; Ferrari M D; Belobrajdic L; Weyrauch R; Varela H
CS Departamento de Bioingenieria, Facultad de Ingenieria, Universidad de la Republica, Montevideo, Uruguay.
SO Revista Argentina de microbiologia, (1994 Jul-Sep) Vol. 26, No. 3, pp. 105-15.
Journal code: 8002834. ISSN: 0325-7541.
CY Argentina
DT Journal; Article; (JOURNAL ARTICLE)

LA English
FS Priority Journals
EM 199503
ED Entered STN: 14 Mar 1995
Last Updated on STN: 3 Mar 2000
Entered Medline: 2 Mar 1995

L12 ANSWER 14 OF 21 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
AN 1993:387102 BIOSIS
DN PREV199396062402
TI Enzymic characterization of murine and human prohormone convertase-1 (mPC1 and hPC1) expressed in mammalian GH-4C-1 cells.
AU Jean, Francois; Basak, Ajoy; Rondeau, Normand; Benjannet, Suzanne; Hendy, Geoffrey N.; Seidah, Nabil G.; Chretien, Michel; Lazure, Claude [Reprint author]
CS Neuropeptides Structure Metabolism Lab., Clinical Res. Inst. Montreal, 110 Pine Ave., W. Montreal, Quebec, Canada H2W 1R7, canada
SO Biochemical Journal, (1993) Vol. 292, No. 3, pp. 891-900.
ISSN: 0264-6021.
DT Article
LA English
ED Entered STN: 23 Aug 1993
Last Updated on STN: 23 Aug 1993

L12 ANSWER 15 OF 21 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
AN 1993:342761 BIOSIS
DN PREV199396039761
TI Phosphorylation of C-terminal domain of RNA polymerase II is not required in basal transcription.
AU Serizawa, Hiroaki; Conaway, Joan Weliky; Conaway, Ronald C.
CS Program Molecular Cell Biology, Oklahoma Med. Res. Foundation, 825 NE 13th Street, Oklahoma City, Oklahoma 73104, USA
SO Nature (London), (1993) Vol. 363, No. 6427, pp. 371-374.
CODEN: NATUAS. ISSN: 0028-0836.
DT Article
LA English
ED Entered STN: 26 Jul 1993
Last Updated on STN: 26 Jul 1993

L12 ANSWER 16 OF 21 MEDLINE on STN DUPLICATE 7
AN 91369212 MEDLINE
DN PubMed ID: 1892395
TI The secretion leader of Mucor pusillus rennin which possesses an artificial Lys-Arg sequence directs the secretion of mature human growth hormone by Saccharomyces cerevisiae.
AU Hiramatsu R; Horinouchi S; Uchida E; Hayakawa T; Beppu T
CS Department of Agricultural Chemistry, University of Tokyo, Japan.
SO Applied and environmental microbiology, (1991 Jul) Vol. 57, No. 7, pp. 2052-6.
Journal code: 7605801. ISSN: 0099-2240.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199110
ED Entered STN: 3 Nov 1991
Last Updated on STN: 3 Mar 2000
Entered Medline: 16 Oct 1991

L12 ANSWER 17 OF 21 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
AN 1991:412805 BIOSIS

DN PREV199192079770; BA92:79770
TI PURIFICATION AND CHARACTERIZATION OF RECOMBINANT HUMAN GROWTH HORMONE EXPRESSED IN SACCHAROMYCES-CEREVISIAE.
AU WON T Y [Reprint author]; JEH H S; KIM C K; CHOI H B; HAN K B; PARK S J
CS LUCKY R AND D CENT, BIOTECHNOL, PO BOX 10, TAEJEON, KOREA
SO Korean Biochemical Journal, (1991) Vol. 24, No. 3, pp. 278-284.
CODEN: KBCJAK. ISSN: 0368-4881.
DT Article
FS BA
LA ENGLISH
ED Entered STN: 11 Sep 1991
Last Updated on STN: 11 Sep 1991

L12 ANSWER 18 OF 21 MEDLINE on STN
AN 91222487 MEDLINE
DN PubMed ID: 1367066
TI Downstream processing of proteins from mammalian cells.
AU Ogez J R; Builder S E
CS Genentech, Inc., South San Francisco, California.
SO Bioprocess technology, (1990) Vol. 10, pp. 393-416. Ref: 35
Journal code: 8601086. ISSN: 0888-7470.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
LA English
FS Biotechnology
EM 199106
ED Entered STN: 9 Aug 1995
Last Updated on STN: 9 Aug 1995
Entered Medline: 12 Jun 1991

L12 ANSWER 19 OF 21 MEDLINE on STN
AN 91022017 MEDLINE
DN PubMed ID: 2220390
TI Anabolic and tissue repair functions of recombinant insulin-like growth factor I.
AU Skottner A; Arrhenius-Nyberg V; Kanje M; Fryklund L
CS Kabi Peptide Hormones, Stockholm, Sweden.
SO Acta paediatrica Scandinavica. Supplement, (1990) Vol. 367, pp. 63-6.
Journal code: 0173166. ISSN: 0300-8843.
CY Sweden
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199011
ED Entered STN: 17 Jan 1991
Last Updated on STN: 17 Jan 1991
Entered Medline: 21 Nov 1990

L12 ANSWER 20 OF 21 EMBASE
AN 90210222 EMBASE
DN 1990210222
TI Anabolic and tissue repair functions of recombinant insulin-like growth factor I.
AU Skottner A.; Arrhenius-Nyberg V.; Kanje M.; Fryklund L.
CS Kabi Peptide Hormones, S-11287 Stockholm, Sweden
SO Acta Paediatrica Scandinavica, Supplement, (1990) Vol. 79, No. 367, pp. 63-66.
ISSN: 0300-8843 CODEN: APSQA7
CY Sweden
DT Journal; Conference Article
FS 003 Endocrinology
007 Pediatrics and Pediatric Surgery

030 Pharmacology
037 Drug Literature Index
LA English
SL English
ED Entered STN: 13 Dec 1991
Last Updated on STN: 13 Dec 1991

L12 ANSWER 21 OF 21 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
AN 1989:501151 BIOSIS
DN PREV198937110810; BR37:110810
TI FIRST CONFERENCE ON ADVANCES IN PURIFICATION OF RECOMBINANT PROTEINS INTERLAKEN SWITZERLAND MARCH 14-17 1989.
AU KAUL R [Reprint author]
CS ASTRA RES CENT INDIA, PB NO 359, MALLESWARAM, BANGALORE 560 003
SO Current Science (Bangalore), (1989) Vol. 58, No. 11, pp. 600-601.
CODEN: CUSCAM. ISSN: 0011-3891.
DT Conference; (Meeting)
Conference; Report; (Meeting Report)
FS BR
LA ENGLISH
ED Entered STN: 7 Nov 1989
Last Updated on STN: 11 Jan 1990

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Korean Biochem. J. (1991), Vol. 24, No. 3, pp. 278~284

Purification and Characterization of Recombinant Human Growth Hormone Expressed in *Saccharomyces cerevisiae*

Teug Yeon Won, Hoon Sung Jeh, Chun Hyung Kim, Hyung Bae Choi, Kyu Beom Han and Soon Jae Park*

Lucky R & D Center, Biotechnology, P.O. Box 10, Taejeon, Korea
(Received March 17, 1991)

Abstract: The recombinant human growth hormone (rHGH) was expressed in *Saccharomyces cerevisiae*. The aggregated rHGH molecules were solubilized by rasing pH of the cell lysates. When pH of the solution was lowered to around 6.5, substantial amount of the contaminating yeast proteins was effectively removed. The rHGH was further purified by successive chromatographic steps including DEAE cellulose, Sephacryl S-200, DE-52, and Phenyl-sepharose. The specific activity of the purified rHGH was 2.7 IU per mg, when assessed by radioreceptor assay. This value was higher than that of pituitary-derived international standard HGH, indicating that rHGH was correctly folded.

Human growth hormone (HGH) is synthesized by the acidophil cells of the anterior pituitary as a prehormone with a hydrophobic leader peptide of some 20 amino acids. The leader peptide is removed by the pituitary during the secretion of HGH. Human growth hormone isolated from pituitary extracts is heterogenous (Gorden *et al.*, 1973; Goodman *et al.*, 1972; Lewis *et al.*, 1980). The major component of HGH is a protein with 191 amino acid residues, with the molecular weight being 22,000 daltons (Li *et al.*, 1964). The minor forms are derived from the major form either by the deletion of 15 amino acid residues (20 kd HGH) (Lewis *et al.*, 1978) or by the deamination of side-chains (Lewis *et al.*, 1979). The native 20 kd HGH is somewhat less active than the native 22 kd HGH in the weight gain assay and the longitudinal bone growth assay (Kostyo *et al.*, 1987). The deaminated forms of pituitary-derived HGH are known to have indistinguishable biological activity as the major 22 kd HGH (Becker *et al.*, 1988).

The three-dimensional structure of HGH is not known yet, although there have been some reports of successful crystallization of recombinant HGH (Jones *et al.*, 1987). However, from the X-ray crystal structure of homologous porcine growth hormone, it is inferred that HGH is also composed of four helical units which make a helical bundle connected by loops (Abdel-Meguid *et al.*, 1987). The protein is stabilized by two disulfide bonds that are easily reduced and re-oxidized. The tetra-S-carbamidomethylated HGH resulting from the reduction, followed by alkylation with iodoacetamide retains all known biological activities in animals and humans (Bewley *et al.*, 1975).

HGH is an hormone that stimulates protein synthesis, lipolysis, and hypoglycemia (Beck *et al.*, 1957). Since growth hormone is species-specific (Knobil *et al.*, 1957), human cadavers had been the only source of HGH to treat hypopituitary dwarfism (Raben, 1959). Recently, HGH has been cloned and expressed in *Escherichia coli* (Goeddel *et al.*, 1979) and *Saccharomyces cerevisiae* (Tokunaga *et al.*, 1985; Cho *et al.*, 1988). These recombinant HGH (rHGH) retained the full range of biological activities (Olson *et al.*, 1981; Park *et al.*, 1990).

Most of rHGH molecules expressed either in bacteria or in yeasts exist as aggregated precipitate. It

*To whom correspondence should be addressed

is therefore not trivial to obtain large quantities of pure rHGH with the correct conformation for therapeutic purposes. In this paper, we describe the purification and characterization of rHGH expressed in *Saccharomyces cerevisiae*.

Materials and Methods

Materials

The *S. cerevisiae* strain transformed with a vector coding HGH for cDNA was provided by Lucky Biotech Corp (Cho *et al.*, 1988). The ADH/GAP promoter was used for the expression of rHGH.

International standard HGH was obtained from NIBSC (National Institute for Biological Standards and Control, U.S.A.). The specific activity of HGH was 2.506 IU/mg according to radioreceptor assay.

C_{18} μ-Bondapak column and Pico. Tag column were purchased from Waters (U.S.A.). DEAE 5PW column was obtained from Tosoh, (Japan).

Phenylisothiocyanate and phenylthiocarbamyl amino acids were purchased from Pierce (U.S.A.). n-Propylalcohol and acetonitrile were obtained from Budick & Jackson (U.S.A.) and Merck (U.S.A.), respectively.

Purification of rHGH

Yeast cells were disrupted with glass beads in distilled water containing 10 mM EDTA and the proteins were extracted by raising the pH to 11.5 with 1 N NaOH. After centrifugation at 11,000×g, the pH of the supernatant was adjusted to 6.5 by dropwise addition of 1% acetic acid. The solution was centrifuged at 11,000×g for 30 min and the supernatant was concentrated with a YM-10 membrane (Amicon). The solution was diluted with 10 mM Tris-HCl at pH 8.0 and concentrated again with a membrane. The solution was then applied to DEAE-cellulose (Whatman DE-52) equilibrated with 10 mM Tris-HCl at pH 8.0. The contaminating proteins were removed from the column by eluting with 50 mM NaCl in the 10 mM Tris-HCl at pH 8.0 and then rHGH was eluted with 100 mM NaCl in 10 mM Tris-HCl at pH 8.0. The protein solution from the DEAE step was chromatographed in Sephacryl S-200 (Pharmacia-LKB) equilibrated with 10 mM Tris-HCl containing 2 M urea at pH 8.0.

The fractions containing rHGH were pooled and then applied to DE-52 equilibrated with 10 mM Tris-HCl, pH 7.5, containing 60 mM NaCl to remove the contaminating materials. The rHGH was eluted from the column by 80 mM NaCl in 10 mM Tris-HCl at pH 8.0. The purity of rHGH was further improved by the use of a Phenyl-sepharose (Pharmacia-LKB) column, in 10 mM Tris-HCl at pH 7.5 with a reverse gradient of NaCl (from 1,000 mM to 0 mM). The purified rHGH was dialyzed against a glycine-phosphate buffer at pH 7.4 and then lyophilized. The purity of rHGH was assessed by silver-staining after 15% (acrylamide:bis) SDS-PAGE (Laemmli, 1970).

HPLC of purified rHGH

The reverse-phase HPLC column used was C_{18} μ-Bondapak (4.5×300 mm), which was equilibrated with 4% (v/v) n-propylalcohol/0.2% phosphoric acid in H_2O. The purified rHGH dissolved in 270 mM glycine-0.6 mM sodium phosphate at pH 7.4 was directly loaded into the column. The concentration of n-propylalcohol was increased linearly to 48% over 30 min and then increased to 78% over 10 min.

For anion-exchange HPLC, the rHGH solution was loaded into a DEAE 5PW-HPLC column (7.8×45 mm) equilibrated with 10 mM Tris-HCl, 30 mM NaCl, at pH 8.0. The salt concentration was increased linearly to 130 mM over 40 min. The elution profile was monitored at 280 nm.

Isolation of the C-terminal fragment

Ten mg of purified rHGH was dissolved in 2 ml of 70% (v/v) formic acid. 10 mg of cyanogen bromide (CNBr) dissolved in 100 μl of 70% formic acid was added. After incubation in the dark for 16 h at 20℃, the mixture was diluted with 5 vol. of ice-cold distilled water to slow down the reaction and then immediately lyophilized. The lyophilizate was dissolved in 2 ml of distilled water and then filtered. The C-terminal fragment was obtained by the reverse-phase HPLC seperation in a C_{18} μ-Bondapak column with a linear gradient of acetonitrile (20-80%) in 0.1% trifluoroacetic acid.

Amino acid composition

Samples were first hydrolyzed in 6 N-HCl (contain-

Table 1. Purification of rHGH

Step	Total[1] protein (g)	rHGH (g)	Specific[2] activity (IU/mg)	Recovery yield (%)
Crude extract(pH 11.5 supernatant)	710	15.3	0.058	100
pH 6.5 supernatant	88.4	13.7	0.42	73
DE-52	18.5	7.3	1.1	48
Sephacryl S-200	5.7	4.1	1.9	27
DE-52	3.8	3.5	2.5	23
Phenyl-sepharose CL-4B	2.8	2.8	2.7	18

[1]Protein concentration was determined by the TCA-Lowry method (Bensadon *et al.*, 1976)
[2]Activity was determined by radioreceptor assay (Park *et al.*, 1990).

ing 1% phenol) for 24 h at 105℃ and then reacted with phenylisothiocyanate (PITC) to produce phenylthiocarbamyl (PTC) amino acids. The amino acid derivatives were analyzed by HPLC using a Pico · Tag column (3.9×150 mm). The peak area of each amino acid was calculated by comparison with the areas of standard PTC-derivatized amino acids, monitored at 254 nm.

Amino acid sequence determination

The amino acid sequences of the twenty six N-terminal residues and the C-terminal fragment from the CNBr digestion were determined by a automated Edman degradation reactions in a Protein Sequencer (Applied Biosystems, Model 471 A, USA). The programs used for the operations of the sequencer were provided by the manufacturer. The phenylthiohydantoin derivatized amino acids were seperated and identified by reverse-phase HPLC.

Radioreceptor assay

The biological potency of the purified rHGH was assessed by radioreceptor assay. The plasma membrane fragments containing the growth hormone receptors were obtained from the liver of pregnant rabbits according to the method described by Tsushima *et al.* (1976). The experimental procedure and the calculation of specific activity of HGH were reported in detail by Park *et al.* (1990).

Results

Purification of rHGH

The majority of rHGH molecules was found in the form of insoluble aggregates in *Saccharomyces cerevisiae*. Those aggregates were readily solubilized in an alkaline buffer after the lysis of the yeast cells. The lowering of pH of the soluble fraction to around 6.5 was an effective step in removing the contaminating yeast proteins (Table 1). When the pH of the solution was lowered to less than 6.0, a substantial amount of rHGH precipitated along with the contaminating proteins. This is due to the limited solubility of rHGH as the pH value of the solution approaches near the pI (5.0) of rHGH.

After the successive chromatographic steps, the purity of rHGH obtained was more than 99% when assessed by silver-staining after SDS-PAGE. As shown in Table 1, the overall recovery yield of rHGH was about 18%. Fig. 1 represents the results of SDS-PAGE at each of the purification steps. The purification scheme used was suitable for the large-scale production of rHGH.

High performance liquid chromatography

The homogeneity of the purified rHGH was tested by applying an aliquot of the final sample to a C_{18} reverse-phase HPLC column. As shown in Fig. 2, rHGH was the only protein detected as n-propanol concentration was increased. When acetonitrile was used as a mobile phase, a similar result was obtained.

In order to test the homogeneity of the protein in another analytical chromatographic system, the sample was applied to a DEAE-HPLC column. As Fig. 3 shows, the majority of rHGH eluted as a single peak followed by an additional minor peak (about 2% of total area). When the fractions corresponding to the minor peak were collected and then subjected

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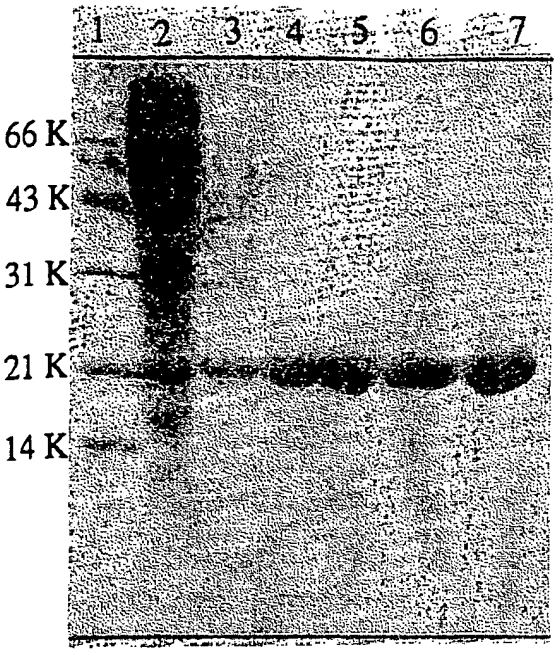


Fig. 1. Analysis of rHGH purification by SDS-PAGE. Lane 1 shows the molecular weight standard. The gel was loaded with aliquots from each of the successive purification steps as follows: crude extract (lane 2); supernatant at pH 6.5 (lane 3); 1st DE-52 pool (lane 4); S-200 gel filtration pool (lane 5); 2nd DE-52 pool (lane 6); Phenyl-sepharose CL-4B pool (lane 7).

to amino acid sequencing, the N-terminal amino acid sequence (up to 10 residues) was identical to that of rHGH from the major peak. Therefore, it is deduced that the minor component may be a deaminated form of rHGH since it is eluted at a higher salt concentration than the major component. The pI value of the minor peak (4.9) was, as expected from the DEAE elution pattern, lower than the value of the major form of rHGH (5.1) (data not shown).

Amino acid sequence identification

In order to confirm that cDNA sequence of rHGH was faithfully translated, the amino acid analysis and the amino acid sequencing were performed with the purified rHGH. The result of the amino acid assay (Table 2) shows that the experimental value for each amino acid agrees well with the values deduced from the cDNA sequence (Fig. 4).

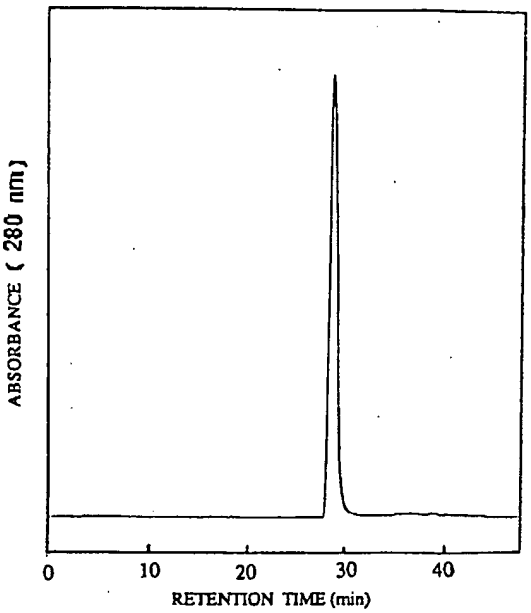


Fig. 2. Reverse-phase HPLC chromatogram of the purified rHGH. Aliquot of the protein solution was applied to a C_{18} μ-Bondapak (4.5×300 mm) HPLC column. The mobile phase used was 4% n-propylalcohol/0.2% phosphoric acid. The flow rate was 0.6 ml/min. The experimental condition was as described in the main text.

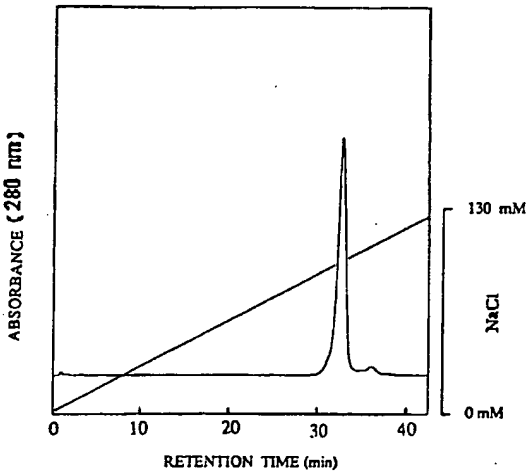


Fig. 3. DEAE-HPLC chromatogram of the purified rHGH. DEAE 5PW-HPLC column was equilibrated with 10 mM Tris-HCl, 30 mM NaCl, pH 8.0. The flow rate was 1.0 ml/min.

Table 2. Amino acid composition of purified rHGH

Amino acid	Experimental values	Theoretical values
Asp/Asn	18.7(19)	20
Glu/Gln	26 (26)	27
Ser	16.3(16)	18
Gly	7.4 (7)	8
His	3.5 (3)	3
Arg	12.3(12)	11
Thr	10 (10)	10
Ala	8.4 (8)	7
Pro	8.5 (8)	8
Tyr	7.4 (7)	8
Val	7 (7)	7
Met	4.8 (5)	4
Cys[1]	2.9 (3)	4
Ile	7.3 (7)	8
Leu	27.5(27)	26
Phe	13 (13)	13
Lys	9.4 (9)	9
Trp	ND[2]	1

[1]Cys residues were not protected prior to the acid hydrolysis.
[2]ND: not determined

The amino acid sequence of the N-terminal 26 residues were identical to the amino acid sequence of the pituitary-derived HGH (Fig. 4). The rHGH from yeast cells has methionine at its N-terminus, which is also the case in rHGH expressed in *E. coli*.

The 21 amino acid fragment of the C-terminus of rHGH was obtained by C_{18} reverse-phase seperation of CNBr-digested rHGH. For the CNBr cleavage of rHGH, disulfide bridges were not reduced nor protected. Hence, the purpose of this experiment was to identify not only the C-terminal amino acid sequences of rHGH, but also the match of disulfide bridges by isolating the cyclic peptide which retains disulfide-bridge between Cys182 and Cys189 (Fig. 4).

The peak marked with the arrow in Fig. 5 indicates the C-terminal 21 amino acid fragment. The amino acid sequence of the fragment was identical to that deduced from the cDNA sequence, except at two positions (182 and 189 as counted from the N-terminus of HGH). At these two positions, the amino acid se-

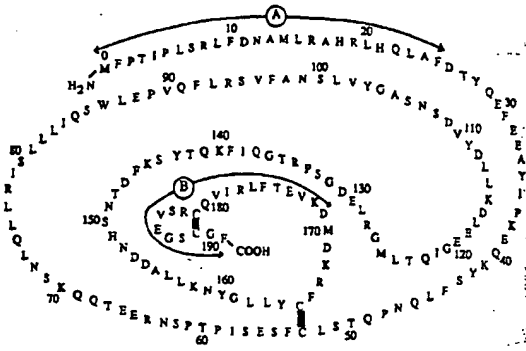


Fig. 4. Amino acid sequence of rHGH. The amino acid sequence of rHGH was deduced from the cDNA sequence. It starts with Met at position 0. The sequences of N-terminal 26 amino acids (A) and C-terminal 21 amino acids (B) were identified by an automated peptide sequencer.

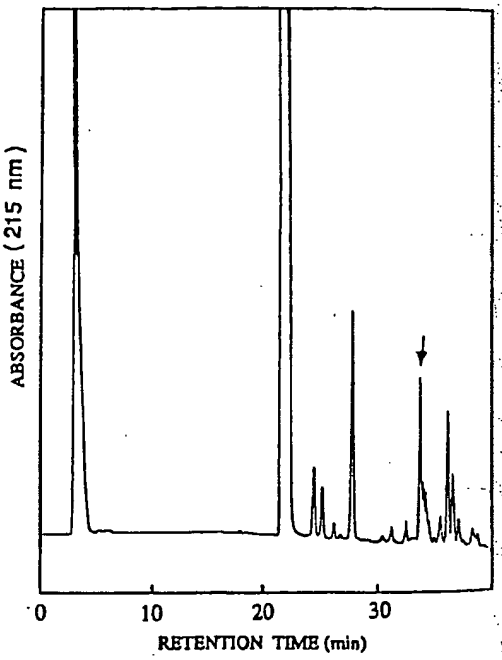
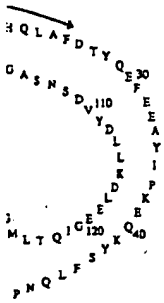


Fig. 5. C_{18} reverse-phase HPLC chromatogram of rHGH cleaved with cyanogen bromide. The lyophilized sample was applied to a C_{18} μ-Bondapak HPLC column with a linear gradient of acetonitrile (20-80%) in 0.1% trifluoroacetic acid. The peak indicated by the arrow is the C-terminal fragment of 21 amino acids. When the elutant was monitored at 280 nm, the peak was not detected due to the absence of aromatic side-chains in the peptide.



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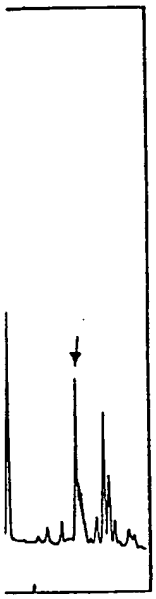

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quence was not detected, suggesting that the amino acid may be a Cys connected to another Cys. This result suggests that the rHGH has the correct match of disulfide-bridges as those of natural HGH.

Discussion

Although the expression of rHGH from *E. coli* has been well established by others, the expression and purification of rHGH from *S. cerevisiae* were not well known. The construction of the vector carrying the cDNA of rHGH for *S. cerevisiae* was reported earlier (Cho *et al.*, 1988). The advantage of using yeast over *E. coli* as an expression host is the extremely low level of endotoxin in the final product. The endotoxin level is especially critical for rHGH compared to other recombinant therapeutic proteins because the amount of protein required per dosage for human injection is much higher for rHGH than, for example, recombinant lymphokines.

The purification method described in this paper is rather simple and therefore can be easily accommodated for the large-scale production of rHGH for clinical use. The protein is extremely pure and the degree of deamination is also low. The DEAE-HPLC pattern of rHGH shown in Fig. 3 is very similar to the one obtained from *E. coli* (Jones *et al.*, 1987). It has been reported that deamination occurs at either Asn149 or Asn152. The deamination of HGH purified from the pituitary as well as from genetically engineered microorganisms is known to be a common phenomenon. However, the desamido-rHGH has the same biological potency as that of the unmodified HGH molecule (Becker *et al.*, 1988).

The methionine residue at the N-terminus of rHGH was not cleaved. This was also observed with rHGH expressed in *E. coli* (Goeddel *et al.*, 1979). However, it is well-known that the presence of N-terminal methionine does not affect the activity of rHGH. The rHGH purified from *S. cerevisiae* remained biologically active. According to the radioreceptor assay, the specific activity of rHGH is 2.7 IU/mg (Table 1). This value is slightly higher than the value obtained with the international standard HGH (2.5 IU/mg). The higher receptor binding activity of rHGH compared to the natural HGH is probably due to the fact that the purified rHGH is extremely pure and homogeneous.

As reported elsewhere (Park *et al.*, 1990), the activities of the purified rHGH and standard pituitary-derived HGH are indistinguishable when compared in the weight gain test with hypophysectomized rats. The circular dichroism pattern of rHGH purified from yeast cells was also the same as that of pituitary-derived HGH (Park *et al.*, 1990).

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초록 : *Saccharomyces cerevisiae*에서 발현시킨 인성장호르몬의 정제 및 특성 확인

원특연 · 제훈성 · 김천형 · 최형배 · 한규범 · 박순재(럭키 바이오텍 연구소)

유전자 재조합 인성장호르몬(rHGH)이 *Saccharomyces cerevisiae*에서 발현되었다. 재조합 인성장호르몬은 발현 후 세포내에서 침전물로 존재한다. 세포 파괴 후 침전된 rHGH를 용해시키기 위하여 용액의 pH를 올리는 방법이 사용되었다. rHGH를 함유한 세포 용해액을 다시 pH 6.5 부근으로 낮추었을 때 대부분의 효모유래 단백질들은 제거되었다. 조 인성장호르몬은 DEAE 셀룰로오즈, 세파크릴 S-200, DE-52, 그리고 페닐-세파로즈 크로마토그래피 방법들을 통하여 정제되었다. 정제된 rHGH를 방사성 수용체 방법을 이용하여 비역가를 측정하였을 때 2.7 IU/mg이었으며, 대조군으로 쓰인 표준품인 뇌하수체 유래 HGH의 경우는 2.5 IU/mg이었다. 이 사실은 재조합 인성장호르몬이 천연형과 같은 3차 구조를 갖는 단백질로 folding되었음을 시사한다.